

In the claims:

Please amend the claims as set forth in the listing of claims below:

1. (Currently Amended) A method for preparing fetal nucleated red blood cells (NRBCs) present in maternal peripheral blood for prenatal genetic investigation, comprising the steps of:

a.) mixing maternal blood, and tissue culture medium, ~~and an aqueous solution containing citric acid, Na citrate and dextran~~, to form a non-physiological tissue culture mixture having the following characteristics:

pH	6.4-6.6	
osmolarity	300-330	mOsm
Na ⁺	150-170	mmol/l
K ⁺	4.5-5.5	mmol/l
Cl ⁻	100-115	mmol/l
Ca ⁺⁺	1.00-2.50	mmol/l
glucose	400-500	mg/dl
lactate	10-20	mg/dl

b) transferring the non-physiological tissue culture mixture obtained in step a) into a cell separation device, followed by introducing into said separation device a liquid having a density higher than maternal blood and containing a red blood cells RBCs (RBCs) aggregating agent,

c) in discontinuous density gradient, subjecting the separation device to centrifugal force to isolate the NRBCs having a lower density than the liquid introduced in step b);

d) washing the isolated NRBCs and resuspending them in tissue culture medium; and

e) ~~identifying fetal NRBCs by appropriate identification procedures and counting~~
ascertaining the presence said fetal NRBCs.

2. (Cancelled)

3. (Previously Presented) The method of claim 1 in which the non-physiological mixture obtained in step a) has the following characteristics:

pH	6.5	
osmolarity	320	mOsm
Na ⁺	165	mmol/l
K ⁺	5.35	mmol/l
Cl ⁻	110	mmol/l
Ca ⁺⁺	1.25	mmol/l
glucose	500	mg/dl
lactate	10	mg/dl.

4. (Original) The method of claim 1 in which the RBCs aggregating agent of step b) is Ficoll.

5. (Original) The method of claim 1 in which the density of the liquid introduced in the separation device by the step b) is 1.068 g/ml.

6. (Previously Presented) The method of claim 1 in which the separation device used in step b), comprises an elongated chamber, whose cross section decreases from the base towards the top, at least a first channel one end of which opens into said chamber near said base and the other end is connected to a pressurized liquid source, and a second channel one end of which opens into the elongated chamber at the device top while the other end opens at the exterior of the device, said device further comprising at

least one additional channel, one end of which opens at a middle level of said chamber height and the other end opens at the exterior of the device.

7. (Cancelled)

8. (Currently Amended) The method of claim 7 1, further comprising counting the identified said fetal NRBCs.

9. (Currently Amended) The method of claim 1, in which the separation device ~~is as illustrated in Figure 4~~ has a base and a top and containing an elongated chamber whose cross section decreases from the base towards the top of the device which contains at least a first channel, one end of which opens on the inside of the chamber near the base and the other end connectable to a pressurized liquid source and a second channel whose end opens in the chamber at a level corresponding to the device top, wherein there is at least a third channel in the device, one end of which opens at an intermediate level of the chamber length, and the other end opens outwards from the device and wherein near the base a flow deflector is provided to disperse evenly through the entire cross section of the inside chamber of the incoming fluid arriving from the first channel.

10. (New) The method of claim 1, wherein the isolation of said NRBCs in step c) is performed in one separation device.

11. (New) A method for separating fetal nucleated red blood cells (NRBCs) from maternal blood cells comprising:

providing peripheral maternal blood comprising nucleated red blood cells (NRBCs) and maternal blood cells having overlapping density distribution profiles;

causing the density of said NRBCs to decrease and the cell density of said maternal

blood cells to increase by transferring said maternal blood into a non-physiological liquid comprising non-physiological tissue culture medium to create a non-physiological tissue culture mixture, wherein said non-physiological tissue culture mixture has a pH of 6.4 to 6.6; and

causing separation of said NRBCs from said maternal blood cells by subjecting said non-physiological tissue culture mixture to centrifugation in a discontinuous density gradient.

12. (New) The method of claim 11, wherein said tissue culture mixture has an osmolarity of 300-330 mOsm.

13. (New) The method of claim 11, wherein said maternal cells are lymphocytes and monocytes.

14. (New) The method of claim 11, wherein a liquid having a density higher than maternal blood and containing red blood cells (RBCs) aggregating agent is added immediately after transferring said maternal blood into a non-physiological liquid comprising non-physiological tissue culture.

15. (New) The method of claim 14, wherein said agent is Ficoll.

16. (New) The method of claim 11, wherein said separation is performed in a single separation device.

17. (New) The method of claim 11, wherein a cellular fraction comprising said NRBCs is transferred into a physiological tissue culture medium and the presence of fetal NRBCs is ascertained.

18. (New) The method of claim 17, wherein the presence of said fetal NRBCs is

ascertained by the presence of ϵ -chain hemoglobin and/or by FISH.

19. (New) The method of claim 18, wherein the presence of ϵ -chain hemoglobin is ascertained by anti- ϵ chain hemoglobin antibodies.

20. (New) The method of claim 1, wherein isolation of NRBCs can be accomplished in a single centrifugation step.

21. (New) The method of claim 11, wherein NRBCs and maternal blood cells can be separated in a single centrifugation step.

22. (New) The method of claim 1, wherein said maternal blood and tissue culture medium is further mixed with an aqueous solution containing citric acid, Na citrate and dextrose, to form said non-physiological tissue culture mixture.

23. (New) A method for separating fetal nucleated red blood cells (NRBCs) from maternal blood cells comprising:

providing peripheral maternal blood comprising nucleated red blood cells (NRBCs) and maternal blood cells having overlapping density distribution profiles;

causing the density of said NRBCs to decrease and the cell density of said maternal blood cells to increase by transferring said maternal blood into a non-physiological liquid comprising non-physiological tissue culture medium to create a non-physiological tissue culture mixture; and

causing separation of said NRBCs from said maternal blood cells by subjecting said non-physiological tissue culture mixture to centrifugation in a discontinuous density gradient,

wherein a separation device having a base and a top and containing an elongated chamber whose cross section decreases from the base towards the top of the device which contains at least a first channel, one end of which opens on the inside of the chamber near the base and the other end connectable to a pressurized liquid source and a second channel whose end opens in the chamber at a level corresponding to the device top, wherein there is at least a third channel in the device, one end of which opens at an intermediate level of the chamber length, and the other end opens outwards from the device and wherein near the base a flow deflector is provided to disperse evenly through the entire cross section of the inside chamber of the incoming fluid arriving from the first channel is used to subject said non-physiological tissue culture mixture to said centrifugation in a discontinuous density gradient.

24. (New) A method for identifying fetal nucleated red blood cells (NRBCs) present in maternal peripheral blood, comprising the steps of:

- a.) providing a non-physiological tissue culture mixture having a pH of 6.4-6.6, wherein said non-physiological culture tissue mixture comprises at least peripheral maternal blood and non-physiological tissue culture medium;
- b) transferring the non-physiological tissue culture mixture of step a) into a cell separation device, followed by introducing into said separation device a liquid having a density higher than maternal blood and containing a red blood cells (RBCs) aggregating agent,
- c) in discontinuous density gradient, subjecting the separation device to centrifugal force to isolate the NRBCs having a lower density than the liquid introduced in step b);
- d) washing the isolated NRBCs and resuspending them in tissue culture medium; and
- e) ascertaining the presence of said fetal NRBCs.